Chapter 1

Introduction
1.1 Introduction to microfluidics

The trend in life science research to miniaturize analytical processes using microchips, first proposed in the late eighties, is ongoing. The idea of integrating sampling, sample handling, reactions, separations and detection into one automated device containing interconnected microchannel networks led to the introduction in the literature of the term Miniaturized Total Analysis System (µTAS) in 1990.¹ In the beginning of the nineties, Manz et al. published papers questioning if miniaturization would be “a look into the next century’s technology or just a fashionable craze?”², ³ The first analytical result achieved with a miniaturized system was reported shortly thereafter, namely the electrophoretic separation of fluorescein and calcein on a glass chip, in a 30-µm-wide, 10-µm-deep and 6.5-cm-long channel.⁴, ⁵ Since that time, the µTAS field has been growing exponentially, as reflected also by the introduction of the more general terms “microfluidics” or “lab-on-a-chip” to describe the technology and its applications. A broad range of applications, including clinical diagnostics, separation sciences, environmental sciences and drug screening, has developed over the past two decades. In particular, the study and analysis of living cells cultivated in microengineered environments which better mimick the in vivo situation promises to generate an unparalleled insight into living systems which will ultimately have an enormous impact on our understanding of life.⁶ Besides the applications developed over the past two decades, the scientific journals, conferences, and companies specializing in lab-on-a-chip technologies are evidence of how interest in this field has grown.

Whitesides defined microfluidics as follows: “It is the science and technology of systems that process or manipulate small (10⁻⁹ to 10⁻¹⁸ liters) amounts of fluids, using channels with dimensions of tens to hundreds of micrometres”.⁷ The small dimensions become advantageous because of the decreased amounts of sample and reagents required for the processes, and the reduction of the time required to perform an analysis and to transport mass and heat. Therefore, the large surface-to-volume ratio of the devices allow for enhanced experimental control of the processes, making microfluidic devices increasingly useful tools for chemists and cell biologists alike.⁸

Figure 1 shows an example of two microfluidic devices made in our laboratory several years ago through replication of microchannels into a silicone rubber followed
by sealing with a glass chip. As the postage stamps indicate, these chips have outer dimensions on the order of a few cm, and thus fit easily into the hand. Another big advantage of the technology can be observed here, that is, the devices are optically transparent. Generally speaking, one can usually look directly into microchannels with a microscope, to observe the behavior of fluids and follow the progress of a reaction or analysis. The channels in this case are 100 μm wide and 100 μm high, approximately equivalent to a human hair, and have volumes on the order of tens to hundreds of nanoliters. If the application demands it, channels can be made much smaller, just a couple of μm or even sub-μm in diameter. Generally, microchannels do not have circular cross-sectional profiles, but are more semi-circular or rectangular in shape. The latter is the case for the devices in Fig. 1. This does influence the fluid mechanics in these devices somewhat when compared to the behavior of solutions in circular capillaries and tubes.

**Fluid flows: Pressure-driven flow**

Fluid flows in micrometer-sized channels can be generated using different mechanisms. In this work, two mechanisms were employed, namely pressure-driven flow (PF) and electro-osmotic flow (EOF). The PF was generated by exploiting a height difference between the fluid columns at the in- and outlet of the channel. There exist several regimes of PF, depending on the flowrate and the dimensions of the conduit through which the fluid flows.

The type of flow one can expect can be predicted using the dimensionless parameter, the Reynolds number, $Re$. The $Re$ represents the balance between the inertial and...
viscous forces in the fluid. The inertial forces are represented by the average linear velocity, \( v \), and the density, \( \rho \), of the fluid. The viscous forces are represented by the dynamic viscosity, \( \eta \), and the characteristic length, \( d \), where \( d \) is the effective diameter of the channel (e.g. diameter of cylindrical channels).\(^9,10\)

\[
Re = \frac{\rho v d}{\eta}
\]  

(1)

When inertial forces dominate and the \( Re \) exceeds 3000 in non-circular tubes (2300 in circular tubes), the flow is called turbulent, and is characterized by a very unpredictable flow pattern. More precisely, the velocity at any one point in the flow is constantly changing, and streamlines are well-defined. However, the characteristic length is small in microchannels, so that the \( Re \) under most normal flow conditions has a value smaller than 10. Flow now falls in the laminar regime, and is characterized by well-defined streamlines. The velocity at the wall is zero, the so-called “no slip” condition, due to the friction between the fluid and the wall, whereas in the center of the channel the maximum velocity is observed. Velocity at any given point in the flow is constant, and flow is very predictable. In this regime, the viscous forces dominate, and the flow can be used for e.g. controlling separations and mixing.\(^10,11\) If the \( Re \) is between 2000 and 3000, the flow is transitional, changing from laminar to turbulent.

**Fluid flows: Electro-osmotic flow**

The second mechanism used in this work to induce flow is EOF. EOF is the bulk motion of solution induced by an externally applied axial electric field\(^12-14\) and originates at the charged surfaces of the substrate, in this case, glass. Due to the use of high-pH buffers, many of the silanol groups at the surface are deprotonated, and the glass bears a negative charge. This leads to the formation of a nm-thick electrical double layer (EDL) containing counter-ions at the channel wall-solution interface. The EDL consists of a stationary inner layer of counter-ions and fluid fixed to the capillary wall, and an outer, more diffuse layer, also containing an excess of counter-ions. Since glass bears a fixed negative charge, the counter-ions in question are cations. When an electric field is applied along the channel, ions in the diffuse part of the EDL will start to migrate, dragging their hydration shells with them, as do all the ions in the channel. However, since there is an excess of cations in the EDL, the net transport of ions in this surface layer is towards the cathode (negative electrode), and a net movement of this surface layer of solution in the same direction results. Motion of the surface solution layer is almost instantaneously transferred to adjacent
solution layers by viscous drag, generating a bulk flow which is characterized by a uniform velocity flow profile across the channel. If we assume that the viscosity, $\eta$, and dielectric constant, $\varepsilon$, of the buffer are the same in the bulk solution and the EDL, the Smoluchowski equation can be used to calculate the electro-osmotic linear velocity, $v_{eo}$, as a function of the applied electric field, $E$. In Equation (2), $\mu_{EOF}$ is the electro-osmotic mobility, $\varepsilon_0$ is vacuum permittivity, and $\zeta_w$ is the zeta potential at the plane of shear in the EDL near the wall.

$$v_{eo} = \mu_{EOF}E = \frac{\varepsilon_0 \varepsilon \zeta_w}{4\pi \eta} E$$

(2)

**Molecular and particle transport by diffusion**

Diffusion is a passive mechanism for molecular and particulate transport, in which a species will move from a region where it is present in high concentration to a region where it is present in low concentration in a random fashion. Diffusion can be mathematically described with Fick’s law,\(^{15}\) and occurs whenever there is a concentration gradient, regardless of the dimensions of the container in which the fluid finds itself. The Einstein-Smoluchowski equation, Equation (3), relates the time, $t$, a molecule needs to diffusion a distance, $d$, to its diffusion coefficient, $D$.

$$d=\sqrt{2Dt}$$

(3)

For a larger molecule or particle, the hydrodynamic radius will influence the diffusion process as a drag force is generated when it is moving with a velocity, $v_p$, through the fluid. This is the Stokes-drag or viscous-drag force, $F$.\(^{16}\)

$$F = f v_p, \quad f = 6\pi \eta r$$

(4)

Here $f$ is the frictional constant and $r$ the radius of the particle. Diffusion is not only time-dependent but also temperature-dependent. The Stokes-Einstein relation, Equation (5) can be used to determine the diffusion constant of particles with known hydrodynamic radii at a given temperature, $T$, using the Boltzmann constant, $k$.\(^{16,17}\) It should be noted that the frictional constant, $f$, for Stokes drag is in the denominator of this equation.

$$D = \frac{kT}{6\pi \eta r}$$

(5)

Now, imagine a 100-µm-wide channel containing a small molecule with a $D$ of $1\times10^{-15}$
Typical molecular diffusion coefficients for macromolecules, like proteins, in water are on the order of $10^{-10}$ m$^2$s$^{-1}$ to $10^{-9}$ m$^2$s$^{-1}$. To diffuse from one side of the channel to the other, the small molecule needs approximately 5 seconds, according to Equation 3. With Equation 5 it can be calculated that a 3-µm-diameter particle in water has a diffusion coefficient on the order of $10^{-13}$ m$^2$s$^{-1}$. It will take 50000 seconds for this particle to diffuse from one side to the other side in the same 100-µm-wide channel. This is much longer than for small molecules.

**Electrophoresis**

Where EOF deals with the motion of the fluid under the influence of an electric field, electrophoresis deals with the movement of charged ions/particles in fluid.\(^{18,19}\) When charged ions start to move under the influence of an electric field they experience Stokes drag. The velocity, $v_{ep}$, becomes:

$$v_{ep} = \frac{qE}{6\pi\eta r}$$

However, for larger particles, the simpler Helmholtz-Smolukowski equation, Equation 7, can be used, since the thickness of the electrical double layer around the particle is relatively thin compared to the particle radius. The EDL dynamics play a reduced role under these conditions. The particle charge can be characterized with the zeta potential, $\zeta_p$, located at the plane of shear close to the solid particle surface.\(^{20}\)

$$v_{ep} = \frac{\zeta_p}{\eta} E$$

**Peclét number**

Due to the predictable flow patterns and the fact that diffusion is a relatively slow process, laminar flow is well suited for the controlled delivery of reagents.\(^{21}\) The Peclét number, $Pe$, relates the diffusive and convective transport in a channel.

$$Pe = \frac{v_d}{D}$$

A large Peclét number is indicative of a flow system in which convective flow dominates, whereas a small Peclét number is characteristic of a system where diffusion is the major mechanism for transport. In microfluidic devices the dimensions, operating conditions and properties of the molecules involved are often known.\(^{11}\) Ho-
However, it depends on the application of the device. Because the small dimensions of the channels and the fact that the diffusion coefficient is small, typical Peclét numbers range between 10 and $10^5$. The diffusion coefficients for particles, e.g. microspheres, cells and DNA, is much smaller than for small molecules. Thus, convective transport dominates and particles may remain in much the same position while being transported by the laminar flow. In other words, the particles will follow the streamline in which they are present, and tend to diffuse only slowly to adjacent streamlines. However, manipulating particles so that they do switch streamlines can be accomplished in various ways, for example by separating particles continuously across a flowing bed of fluid, as reviewed by Pamme.

1.2 Introduction to microfabrication

Microfluidic devices are fabricated with technologies first developed for the microelectronics industry over four decades ago, and used to process microelectromechanical systems (MEMS). The material and type of device determines which processes are chosen for the fabrication. In this section, the techniques used in this research to fabricate microfluidic glass devices are described. Figure 2 gives a schematic summary of the fabrication process.

It all starts with the idea of the channel layout, which is drawn with Computer Aided Design (CAD) software as a 2D plot. The 2D design is printed on a transparency sheet with a high-resolution printer (resolutions of ± 2 to 7 μm, depending on printer and transparency). It may also be “written” into a thin layer of photoresist, a photosensitive polymer coated onto a chromium-covered glass-plate using a laser beam (resolution about ± 1 μm). The resist is then developed, exposing the Cr layer in regions where the mask will need to let light through. The Cr is then removed by chemical etching in these regions. This sheet of glass-plate serves as the optical mask during the photolithographic process. The design on the mask is transferred with UV light into a layer of photoresist, covering a Cr layer deposited on a glass-plate. The UV light is collimated to obtain an exact copy of the mask, that is, with the highest possible resolution, in the photoresist. The illuminated areas are developed using a developer solution and the Cr layer under the resist is exposed in these areas. The Cr layer is then etched away in the areas where it was uncovered during development, to reveal the glass surface underneath. The 2D pattern of the device is then isotropi-
cally etched in the glass with a hydrofluoric-acid etch solution. With isotropic etching, the glass is etched in all directions with the same velocity, resulting in typical D-shaped channels.

The photoresist and Cr layers are now removed and a glass coverplate is prepared to seal the channels. This glass plate is covered with photoresist to protect the surface while making in- and outlets using a powder-blaster. The powder-blaster generates an airstream with µm-sized particles, Al$_2$O$_3$. The particles hit the surface, producing small cavities; as there are many particles present in the airstream, a hole through the plate is eventually produced. The photoresist is removed from the cover-plate and both plates are cleaned. Cleaning is an important factor for successful bonding, together with oxidation of the glass.$^{11}$ After oxidizing with a piranha solution (mixture of hydrogen peroxide and sulfuric acid), the plates are brought together and aligned with one another by positioning in- and outlet holes in the coverplate with the ends of the channel in the other plate. The two plates are bonded together using a process.

Figure 2. Schematic overview of glass device fabrication. (Top) photolithographic transfer using UV-light of the device design on the mask into the photoresist layer coating a chromium-covered glass wafer. After development of the photoresist, the chromium and glass are etched with chromium etch and hydrofluoric acid, respectively. In practice, the mask is in contact with the wafer during exposure. (Bottom) Microfluidic channels after fusion bonding with a second glass plate containing holes for the in- and outlets, and a close-up of the isotropically etched D-shaped channel.
called fusion bonding. They are heated up to approximately 650°C Celsius using a temperature ramp and held at this high temperature to fuse together. This was done with weights placed on top, to apply pressure during the bonding process. A slow cooling process is used to prevent thermal stress and any resulting cracking or breaking of the glass. Figure 3 gives an overview of the device as part of the experimental setup.

![Figure 3. Photograph of the device as part of the experimental setup. The device is placed on an inverted fluorescence microscope. Pipette tips are glued over the in- and outlets to serve as fluid reservoirs. Platinum wires (0.5 mm diameter) inserted into the sides of the reservoirs just above the chip surface served as electrodes to apply the electric field.]

**1.3 Applications of microfluidics**

Since the early days of μTAS, the developments in the field have made it increasingly possible to work with complex fluids like blood, perform single-cell analysis and generate tools which can be used for e.g. clinical or point-of-care diagnostics. Manz has published a series of reviews in collaboration with different authors in which the growing number of microfluidics papers are categorized according to theoretical aspects, technological developments, analytical operations and applications. The analytical operations include on-chip sample preparation, injection, fluid and particle handling, reactors and mixers, separation and detection. The applications sections give an nice overview of possibilities for cell culture and cell handling, clinical diagnostics, immunoassays, proteins, DNA handling techniques (like separation, analysis, polymerase chain reaction and sequencing) and environmental applications. Toner discussed how on-chip sample preparation and analysis of the complex body fluid, blood, can be performed. These reviews are just a few of the many reviews which have recently appeared, giving a nice overview of the current state-of-the-art. The
reader is referred to these for more detailed information regarding the many applications of lab-on-a-chip technology.

1.4 Scope of the thesis

This thesis describes a new, microfluidic, particle separation system based on a particle-trapping phenomenon in continuous flow which requires no physical barriers. The trapping phenomenon, which we have termed *Flow-Induced Electrokinetic Trapping (FIET)*, is the result of two effects, namely a hydrodynamic and an electrokinetic effect. Pressure-driven (PF) and electro-osmotic flows (EOF) are opposed in a narrow glass microchannel that expands at both ends. The resulting bidirectional flow in the narrow channel generates the hydrodynamic effect, a recirculating flow. The second effect is the electrokinetic migration of the particles themselves. It was shown that polymer microspheres can be captured and preconcentrated exploiting FIET. Importantly, the electrophoretic mobility (zeta potential) of the particles determines the flow conditions required for trapping. In this research, FIET is exploited to separate particles.

The first chapter (Chapter 1) gives an introduction to microfluidics and the consequences of working at the microscale. The fabrication process for glass microchannels involved in this work are described, including standard photolithography, wet etching and fusion bonding.

Since microfluidic devices have become more multifunctional tools over the past few years, the ability to work with samples containing particles has also improved. Integration of a step to remove particulate matter from samples may be required for sample preparation, particles may be required to interact with the analyte, or particles may be the actual analyte of interest, requiring sorting or separation. In any case, the approach chosen strongly depends on the application being considered and the properties of the particles themselves. As a result, there are a growing number of examples of particle separation devices in the literature. Chapter 2 gives an overview of current literature on particle separation methods inspired by well-defined laminar flows. The mechanisms discussed are based on 1) pure flow effects, 2) specially designed channels and/or obstacle arrays to generate different streamlines and induce secondary flow effects, or 3) separating particles based on their susceptibility to ex-
ternal acoustic or magnetic forces. Furthermore, an overview of the applications of these mechanisms to biological particles is given.

**Chapter 3** gives a thorough theoretical analysis of the flow mechanisms and the electrokinetic effects involved in FIET. The separation was studied using simple mixtures of two polystyrene bead types having the same size (3 μm) but different charges (zeta potentials). Using a non-uniform channel, one type of particle can be trapped according to its zeta-potential, while particles with higher or lower zeta-potentials are flushed away with the pressure-driven or electro-osmotic components, respectively, of the flow. To gain more insight into the separation mechanism, particle separations in a straight microchannel with uniform cross-section were also studied under conditions of bi-directional flow without the possibility of trapping.

The FIET-system can also be exploited to separate micrometer-sized particles with different sizes, but similar charge. As particles have similar charge (similar zeta potential), they experience the same electrophoretic mobility, but they can be separated in the bidirectional recirculating flow by a mechanism which strongly resembles that of hydrodynamic chromatography (HDC). However, while HDC in its chip form requires relatively long microchannels for separation of sub-μm species, our approach allows separation of larger particles with very good resolution in channels which are just a few mm long. **Chapter 4** addresses this size-based particle separation mechanism, which we have called “tunable hydrodynamic chromatography” for reasons explained in the chapter, and shows results employing FIET in continuous and batch operating modes.

**Chapter 5**, the last research chapter of this thesis, focuses on the application of FIET to the handling and manipulation of biological particles. Three different types of biological particles were used, namely yeast cells, diluted whole blood and two DNA strands of different lengths. These proof-of-principle studies show the potential of FIET for on-chip sample pretreatment, preconcentration and separation of biological particles.

**Chapter 6**, the last chapter, gives a summary of the results. As many interesting questions remain unanswered, suggestions and ideas for future work are also given.
1.5 References

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